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METHOD FOR REVERSIBLE, SITE-SPECIFIC, HIGHLY EFFICIENT AND SIMULTANEOUS IMMOBILIZATION OF DIFFERENT (BIOLOGICAL) MACROMOLECULES ON SOLID PHASES

[VERFAHREN ZUR REVERSIBLEN, ORTSPEZIFISCHEN, HOCHEFFIZIENTEN UND GLEICHZEITIGEN IMMOBILISIERUNG VERSCHEIDENARTIGER (BIOLOGISCHER) MAKROMOLEKUELE AUF FESTPHASEN]

CHRISTOF NIEMEYER, et al.

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INVENTORS (72): CHRISTOF NIEMEYER, LARISSA BOLDT, and DIETMAR BLOHM

APPLICANT (71): CHRISTOF NIEMEYER

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Description

Summary of the Invention

A method for reversible, site-specific, and simultaneous immobilization of different (biological) macromolecules and solid phases.

Background of the Invention

The production of biosensors requires surfaces that are modified in an orderly way with functions macromolecules, frequently biomolecular components, such as, for example, antibodies, enzymes, or receptors. In this case in particular for the development of miniaturized, massively parallel analysis process e.g. within the framework of genome and proteome research, biomedical diagnosis, or active ingredient searching, immobilization methods necessary, that permit functionalization of laterally microstructured surfaces in addition to with nucleic acids as with other macromolecules. In this case methods of reversible immobilization, that make it possible to regenerate configured elements in order to be able to use cost-intensive sensor surfaces several times, are particularly attractive. Former strategies for reversible immobilization of proteins use reversible chemical processes [Tyagi et al. (1994) Process. Biochem., 29, 443], such as, for example chelating metal ions [Anspach, et al. (1994) Biotechnol. Appl. Biochem., 20 323], splitting disulfides [Batistaviera et al. (1991) Appl. Biochem. Biotech., 31, 175], and protein ligand interactions [Phelps et al. (1994) Biotechnol. Progr. , 10, 433], but are not suited for immobilizing different components of a solid phase simultaneously at predetermined sites because of insufficient specificity. The site-specific immobilization of

proteins and other biomolecules required for producing miniaturized parallel sensors can also be achieved by photolithographic methods [Rozsnyai et al. (1992) Angew. Chem. int Ed. Engl., 31, 759]. However, a basic problem in the case of producing high-quality functionalized surfaces results from the poor physical chemical stability of many biological macromolecules. Therefore, successive immobilization methods, that, for example, are used in producing nucleic acid microarrays [Lockhart et al. (1996) Nature Biotechnology, 14, 1675], are not suitable. Instead of this, it is necessary to have a method that permits simultaneously immobilizing many different macromolecules in a single reaction step at specific places on a structured surface with high efficiency.

Solution

This problem is solved by the method named in Patent Claim 1, in which nucleic acids are used as immobilization-mediating reagents and the components to be immobilized are coupled with nucleic acids and the solid phases are functionalized with nucleic acids complementary thereto. The advantages of the method consist in the fact that arbitrarily many macromolecular components, that are connected with an individual, immobilization-mediating sequence, according to Patent Claim 15 can be immobilized in parallel in one reaction step, when complete or half-finished DNA microarrays are used as the solid phase.

An advantageous configuration of the invention is given in Patent Claim 11, in which special nuclei acid-containing adapter molecules are used for coupling the components to be immobilized with the immobilization-mediating nucleic acid. In this way lengthy, direct coupling steps with the immobilization-mediating nucleic acid take

place. Instead of this, only an introduction of a group binding to the adapter molecule is to be carried out under careful reaction conditions.

A further advantageous configuration of the invention is given in Patent Claim 14, in which BIAcore Sensorchips or other commercial products are used as the solid phase. The method named in Patent Claim 1 makes it possible to regenerate these expensive commercial products, which makes them accessible to multiple uses.

Specific Embodiments

Example 1

Parallel Immobilization of Several Enzymes

Several enzymes that may be used according to known methods for biosensorial purposes, for example lipases, dehydrogenases, hydrolases, phosphatases, or other enzymes in each case are coupled with an individual nucleic acid. The coupling is performed covalently with chemical methods or non-covalently with the use of nucleic acid adapter molecules. The nucleic acid-coupled enzymes are mixed in an aqueous buffer and a DNA micro-array that contains surface-bonded nucleic acid fragments complementary to the enzyme conjugates, is introduced into this solution. Because of the site-specificity of the method named in Patent Claim 1, all enzyme components are arranged on the positions on the surface established for them by the array nucleic acids. The protein array thus built up can be used for massively parallel bisensorial purposes, for example detecting environmental poisons, checking the metabolite level of living beings, or for similar tasks.

Example 2

Parallel Immobilization of Several Binding Proteins

Several proteins usable according to known methods for immunological purposes, for example antibodies, receptors, or other binding proteins in each case are coupled with an individual nucleic acid. The coupling takes place covalently with chemical methods or non-covalently with the use of nucleic acid adapter molecules. The nucleic acid coupled binding proteins are mixed in an aqueous buffer and a DNA microarray, that contains the surface bonded nucleic acid fragments complementary to the binding protein conjugates is introduced into this solution. Because of the site-specificity of the method named in Patent Claim 1, all binding protein components are arranged on positions on the surface established by the array nucleic acids. The binding array thus built up can be used for massively parallel, sensorial methods, for example in immunological and medical diagnostics, monitoring metabolites, or for similar purposes.

Example 3

Parallel Immobilization of Synthesis Enzymes

Several enzymes that may be used according to known methods for synthetic purposes, for example lipases, dehydrogenases, hydrolases, phosphatases, glycosidases, aldolases, or other enzymes in each case are coupled with an individual nucleic acid. The coupling is performed covalently with chemical methods or non-covalently with the use of nucleic acid adapter molecules. The nucleic acid-coupled enzymes are mixed in an aqueous buffer and a nucleic acid functionalized solid phase, on which a DNA micro-array, that contains surface-bonded nucleic acid fragments complementary to the enzyme conjugates, is introduced into this solution. Because of the site

specificity of the method named in Patent Claim 1, all enzyme components are arranged at the positions on the surface established by the array nucleic acids. For example, the solid phase functionalized with synthesis enzymes in this way can be integrated in enzyme reaction vessels produced according to known, e. g. microsystem-technology methods and used for synthesizing purposes, for example the production of pharmaceutical effective substances, pure chemicals, or other commercially interesting intermediate and final products of the preparatory synthesis.

Example 4

Use of Adapter Molecules for Coupling Components to be Immobilized with the Immobilization-Mediating Nucleic Acid

Nucleic acid adapter molecules are used in order to perform the coupling of the components to be immobilized with single-strand nucleic acids experimentally in a simple way. In addition to a specific nucleic acid consequence, the adapters have one or more additional bonding places for a chemical group that is introduced into components to be immobilized in a connected reaction. The adapter molecules are typically conjugates of DNA or PNA (protein-nucleic acid)-oligonucleotides and the biotin-binding proteins streptavidin or avidin, respectively derivatives of these proteins. The introduction of biotin into the components to be immobilized takes place under mild chemical conditions with known methods, for example by using the commercially available derivation reagent N-hydroxysuccinimidyl biotin. Then the biotinylated components to be immobilized are mixed with the DNA streptavidin adapter molecule, by which the component is coupled with a specific nucleic acid sequence. It is particularly

advantageous that different adapter molecules in each case can be stored with special nucleotide sequences, for example as stock solutions, so that any biotin-containing components can be coupled with individual nucleic acid sequences according to the modular principle.

Example 5

Regeneration of Sensor Surfaces

The surface of a sensor chip, for example commercially-available streptavidin-coated gold sensor chips for the BIAcore-biosensor (e.g. BIAcore SAS-chips) based on plasmon resonance, is coated with nucleic acid fragments according to known methods. A biotin-derived antibody (immunoglobulin G, IgG) is mixed with an adapter molecule of single-strand DNA and streptavidin, the IgG being coupled with the immobilization-mediating nucleic acid. The resulting conjugate is injected via the sensor chip and binds to the chip surface by nucleic acid hybridization. The sensor chip functionalized in this way with antibodies can be used for sensor measurements to be carried out according to known methods, for example measurement of protein-protein interactions. If the antibody binding capacity is created on the basis of the naturally appearing denaturing of the protein, the antibody nucleic acid conjugate is typically solved by treatment with aqueous soda lye or heat treatment. Since the nucleic acid coating of the sensor chip survives these treatments without harm, a new antibody nucleic acid conjugate can be immobilized by hybridization on the chip, whereupon the sensor chip can be used for further interaction measurements. This regeneration step typically can be repeated more than 100 times because of the high physical-chemical stability of many

nucleic acids.

Summary

A method for reversible, site-specific, highly efficient and simultaneous immobilization of different (biological) macromolecules on solid phases. The macromolecular components to be immobilized, for example proteins, nucleic acids, cell components and organelles, microorganisms, cells, and also organic and inorganic particles such as chromophores and fluorophores, peptides, metal and semimetal clusters, vesicles, or membranes, are coupled with immobilization-mediating nucleic acids, or preferably with nucleic acid-containing adapter reagents, so that the macromolecules then can hybridize on complementary, solid phase-fixed nucleic acids. The surfaces functionalized by means of nucleic acid hybridization are suited as solid phases for miniaturized biosensors, bioreagents, and other functional elements capable of being regenerated.

Example 6

Use of Non-natural Nucleic Acid Analogs as Immobilization Mediators

Several different macromolecules, for example enzymes, chromophores, metal colloids, vesicles, or any other components in each case are coupled with an individual nucleic acid analog. The coupling takes place covalently with chemical methods or non-covalently with the use of adapter molecules. According to Patent Claim 4, nucleic acid analogs that are produced by known methods [Collins et al. (1997) Nucl. Acids Res. 25, 2979] and that do not bind to native nucleic acids are used as immobilization-mediators. The use of nucleic acid analogs takes place in the method named in Patent Claim 1, in order to prevent a non-specific binding, for example RNA

and DNA components present in cell extracts and, instead of this, to exclusively attach the macromolecules to be immobilized on the solid phase.

Example 7

Immobilization of Organic and Inorganic Materials on Nucleic Acid Arrays

Different inorganic and organic components, for example the biomolecules, organic and inorganic macromolecules named in Patent Claims 5-7 are modified with nucleic acid immobilization-mediators and immobilized on a nucleic acid microarray. An arrangement of different material components arises as a result of the immobilization of the individual components. This arrangement can be used, among other things, by means of known methods for further separation of materials, for example by reductively precipitating silver on immobilized gold colloids [Holgate et al. (1983) Histochem. Cytochem. 31, 938]. The arrangements of material components produced by the method named in Patent Claim 1, for example can be used as a functional unit in nanotechnical or microsystem-technical devices.

Patent Claims

1. A method for reversible, parallel, site-specific and highly efficient immobilization of macromolecules on solid phases, **wherein** nucleic acids are used as immobilization-mediating reagents.

2. The method according to Claim 1, wherein the components to be immobilized are coupled with nucleic acids and the solid phases are functionalized with nucleic acids complementary to them.

3. The method according to Claim 1, wherein desoxyribonucleic acids or ribonucleic acids are used as immobilization mediators.

4. The method according to Claim 3, wherein modified nucleic acids such as peptide nucleic acids, phosphothioate, other synthetic molecules with a specific recognition capacity for surface-bonded biomolecules are used as immobilization mediators.

5. The method according to Claim 1, wherein the components to be immobilized are biological macromolecules, such as antibodies, enzymes, receptors, membrane proteins, glycoproteins, carbohydrates, nucleic acids, or other biomolecules.

6. The method according to Claim 1, wherein the components to be immobilized are inorganic chemical components, such as metal and semimetal clusters.

7. The method according to Claim 1, wherein the components to be immobilized are organic chemical groups having specific catalytic, optical, or electric properties.

8. The method according to Claim 1, wherein a surface is functionalized with nucleic acids, so that different components modified with complementary nucleic acids can be bonded.

9. The method according to Claim 1, wherein the components to be immobilized are covalently coupled with the immobilization-mediating nucleic acid are coupled.

10. The method according to Claim 1, wherein the components to be immobilized are non-covalently coupled with the immobilization-meditating nucleic acid.

11. The method according to Claim 10, wherein nucleic acid-containing adapter molecules are used for coupling the components to be immobilized with the immobilization-mediating nucleic acid.

12. The method according to Claim 11, wherein conjugates of

streptavidin, avidin, or recombinant or chemically modified derivatives of these proteins and single-strand nucleic acids are used as adapter molecules.

13. The method according to Claim 11, wherein conjugates of antibodies, receptors, or other binding proteins and single strand nucleic acids are used as adapter molecules.

14. The method according to Claim 11, wherein BIAcore Sensorchips or other commercial products are used as the solid phase.

15. The method according to Claim 11, wherein complete or half-finished DNA microarrays are used as the solid phase.

16. The method according to Claim 11, wherein functionalized solid phases are produced for sensor elements.

17. The method according to Claim 11, wherein functionalized solid phases are produced for reactor elements.

18. The method according to Claim 11, wherein functionalized solid phases are produced for elements with electric, electronic, or optical functions.